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Note

Longevity of neutralizing antibody levels in macaques vaccinated with Quil A-adjuvanted measles vaccine candidates

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Abstract

Quil A-based candidate measles vaccines have been shown to be immunogenic and protective in cotton rats and macaques. Here we studied the longevity of protective VN antibody levels induced in macaques with one dose of measles virus (MV) iscom. Inactivated MV adjuvanted with iscom-matrix or with purified *Quillaja* saponin QA-22 were also tested. All animals developed high levels of VN antibody and MV-specific IFNγ-producing cells. Especially the high VN antibody levels induced by the latter two preparations showed virtually no decrease during the 2-year follow-up. These highly promising candidate MV vaccines should now be tested in infant macaques in the presence or absence of passively transferred and/or maternally derived VN antibodies. In addition, the immunopathological safety of the constructs should be evaluated in the atypical measles model in rhesus macaques.

Keywords: Measles; Iscom; Quil A

The possibility to induce long-term protection against measles with a "one-shot—early in life" vaccination approach, would be a major step forward towards measles control and the eventual eradication of measles virus (MV) [1]. Recently, we showed that both iscom- and modified vaccinia virus Ankara (MVA)-based candidate measles vaccines may induce protection against MV infection in macaques, when administered in the presence of pre-existing virus neutralizing (VN) serum antibody levels that preclude successful vaccination of infants with live attenuated vaccines (LAV) [2–4]. It has been shown that VN antibody levels ≥0.2 international units (IU)/ml are protective in infants and macaques [2,3].

The development of Quil A-based vaccines has been hampered by problems related to toxicity of crude Quil A preparations. The safety of iscom- and Quil A component-based vaccines has largely been improved, since with highly purified Quil A components virtually non-reactogenic vaccines can be made [5–7]. Further identification of components and structures essential for the induction of an optimal immune response with MV-iscom, have focused on the use of different purified Quil A components [8]. In addition, the question was addressed whether formation of the characteristic iscom structure and the incorporation of antigen into this structure are required to induce cytotoxic T lymphocytes (CTL) in vitro and protective immune responses in vivo [8]. On the basis of the data generated in these studies three different preparations were selected for further evaluation: a MV-iscom preparation, β-propiolactone (BPL)-inactivated MV adjuvanted with iscom-matrix and BPL-inactivated MV adjuvanted with QA-22 [8]. These three candidate vaccines induced protection against challenge with wild-type MV in cotton rats [9]. These results prompted us to evaluate the longevity of protective levels of MV neutralizing antibody in macaques induced by one dose of these Quil A-based vaccine candidates. To this end, a vaccination study was carried out in 15 captive-bred subadult healthy cynomolgus monkeys (*Macaca fascicularis*). Preparation and characterization of the three vaccine candidates were described previously [8]. Briefly, MV-iscoms were prepared using the "dialysis method" with HPLC-purified Quil A components QA-3 and QA-22 (ratio 1:4). The iscom-matrixes were prepared identically to the MV-iscoms with the omission of solubilized MV. The iscom-matrix and "free" QA-22 were mixed with BPL-inactivated MV so that the F-protein-to-Quil A ratio was 0.5. The macaques were vaccinated intramuscularly with one dose of the vaccines (five animals per group),

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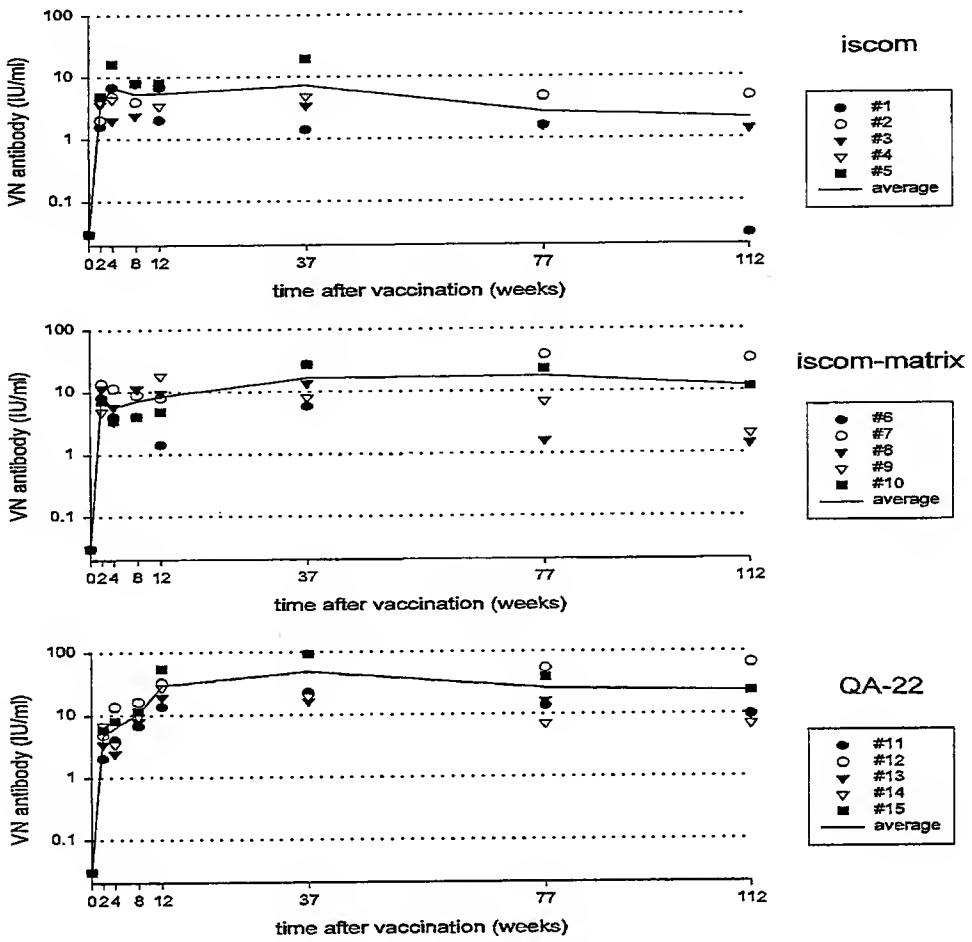

Fig. 1. Development of MV-specific virus neutralizing antibodies in plasma collected at different time-points after vaccination. Macaques were vaccinated intramuscularly at week 0 with one dose of MV-iscom preparation (#1–5), BPL-inactivated MV adjuvanted with iscom-matrix (#6–10) or BPL-inactivated MV adjuvanted with QA-22 (#11–15). Each dose of each preparation contained 10 μg F-protein and a F-protein-to-Quil A ratio of 0.5. The line represents the average VN antibody level of the group.

each containing 10 μg F-protein. Heparinized blood samples were collected at weeks 0, 2, 4, 8, 12, 37, 77 and 112, and both plasma and PBMC samples were cryopreserved.

The titers of MV-specific neutralizing antibody in plasma were measured and expressed in IU per ml as previously described [4]. High VN antibody levels (2–16 IU/ml) were induced within the first weeks after vaccination with all three vaccine candidates (Fig. 1). The groups vaccinated with inactivated MV adjuvanted with iscom-matrix and adjuvanted with QA-22 both showed a tendency of higher antibody levels that also persisted at higher levels than those of the MV-iscom group: the former two groups showed virtually no decrease in antibody levels during the 2-year follow-up period.

MV-specific cellular immune responses were measured in a selection of the vaccinated macaques 4 weeks after vaccination using an interferon gamma (IFNγ) enzyme-linked immunospot (ELISPOT) assay for macaques (U-cytech, Utrecht, The Netherlands). PBMC were plated in 96-well V-bottomed plates (Greiner Labor Technik, Nürtingen, Germany) at a concentration of 1.5×10^5 cells per well. To these wells autologous herpes papio virus-transformed B cells, either or not infected with MV Edmonston 48 h before, were added at a concentration of 3×10^4 cells per well. Plates were centrifuged for 10 s, incubated at 37 °C for 1 h and the co-cultured cells were then transferred to the ELISPOT plates (Silent Screen Plate 96-well w/Nylon, Lalge Nunc International, USA). After 6 h cells were removed and the plates processed according to instructions provided by the manufacturer. In all the macaques tested, MV-specific IFNγ-producing cells were demonstrated in PBMC (57–500 per 150,000 cells) collected 4 weeks after the vaccination, with no significant differences between the groups (Fig. 2).

In conclusion, all three measles vaccine candidates tested induced long-lasting protective levels of VN antibody in a "one-shot" regimen. Especially the two vaccines based on inactivated whole MV, adjuvanted either with iscom-matrix or with QA-22, induced antibody levels that showed virtually no decline over the 2-year follow-up period. This would

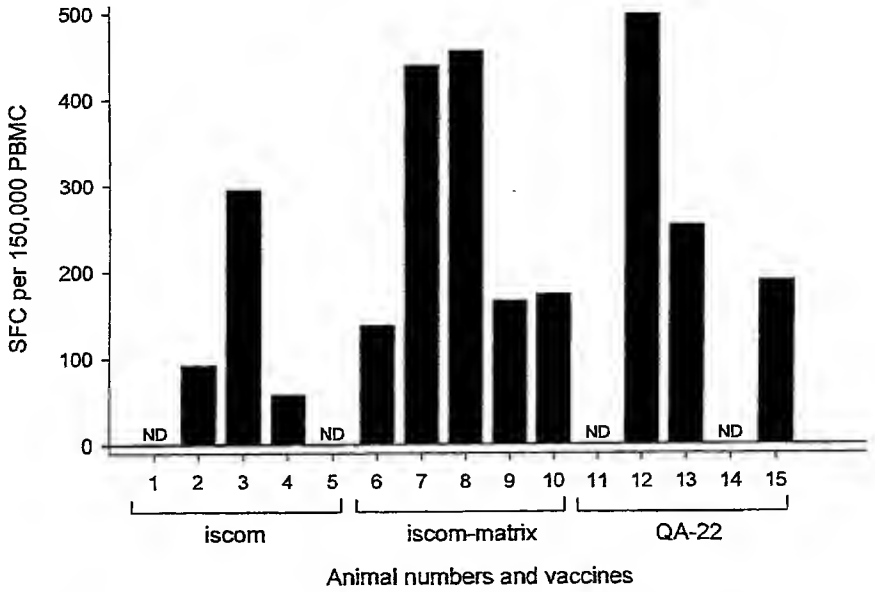

Fig. 2. MV-specific vaccine-induced spot forming cells (SFC) in PBMC at 4 weeks after vaccination as measured by an IFN-γ-specific ELISPOT assay. PBMC were co-cultured during 6 h with herpes papio virus-transformed autologous B cells that were infected with MV. The results were corrected for SFC observed in wells with uninfected herpes papio virus-transformed B cells. PBMC were isolated from macaques that were vaccinated with one dose of MV-iscom preparation (#1–5), BPL-inactivated MV adjuvanted with iscom-matrix (#6–10) or BPL-inactivated MV adjuvanted with QA-22 (#11–15). Each dose of each preparation contained 10 μg F-protein and a F-protein-to-Quil A ratio of 0.5. ND, not done.

favor these two preparations for further efficacy and safety evaluation in infant macaques. These studies should be performed both in the absence and presence of passively transferred and/or maternally derived VN antibodies.

A specific problem with the use of measles vaccines based on inactivated MV, as proposed here, is the legacy of the problem that arose in the late 1960s: the use of formalin-inactivated measles adjuvanted with alum predisposed infants for developing atypical measles upon later infection with wild-type MV [10]. Therefore, before clinical trials can be considered, the safety of these Quil A-based candidate measles vaccines with respect to immunopathology should be addressed in the recently described atypical measles model in rhesus macaques [11].

Taken together, the data generated in this study justify further evaluation of the safety and efficacy of iscom-matrix- and QA-22-adjuvanted measles vaccine candidates.

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